Transfer of PAHs and PBDEs from feed to animals

Feeding Fats safety European Program (FOOD-CT-2004-007020)

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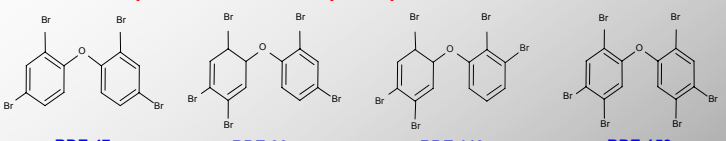
Polybrominated Diphenyl Ethers (PBDEs)



BDE 47 BDE 99 BDE 119 BDE 153

- Flame retardants
  - Polymers for textile backing, electrical & electronic equipment
  - Plastic housings esp. office equipment
  - Cushions; mattresses; carpet padding

- commonly used (209 congeners)
- chemically similar to PCBs
- Toxicology:
  - Nervous system toxicity
  - Reproductive and developmental disruption
  - Endocrine disruption

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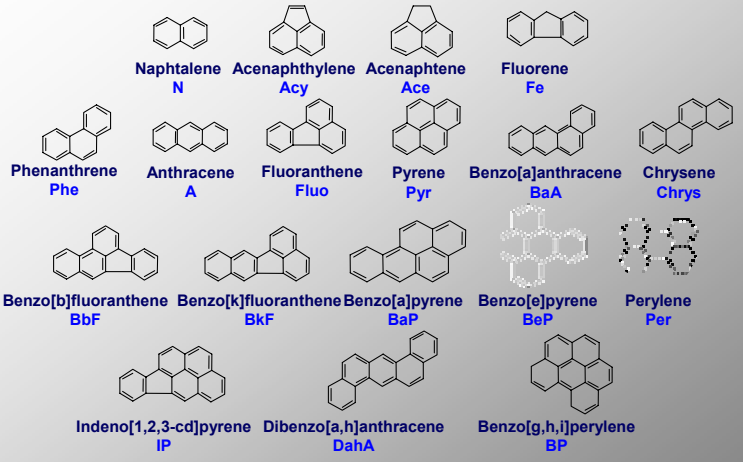
Polycyclic Aromatic Hydrocarbons (PAHs)



- EPA priority pollutants; ubiquitous, persistent, lipophilic;
- Biological activity : carcinogenic and mutagenic for some of them
- BaP : commonly used as a marker of PAH in food (highest carcinogenic potential) …but highly controversial now!

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Origins of PAHs

Mainly **anthropogenic compounds** → 2 principal sources :

Pyrolytic Origin

Transport discharges

Industrial effluents

Atmospheric deposition

Petrogenic Origin

Petroleum activites
(exploitation, transport, oil spill …)

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PAH sources in food

Plants / vegetables : 3 possible sources of contamination : uptake as a result of atmospheric exposure, uptake from the soil, and endogenous biosynthesis, but most of the crop contamination is the result of atmospheric deposition (small airborne particles) → importance of growing area

Grains and raw products for oil production : contamination of seeds during artificial drying and heating during processing (the prominent sources for the contamination of edible oils with PAHs)

Benzo[a]pyrene (BaP) as marker of PAH contamination in food :

BaP (highest carcinogenic potential) largely used as marker of PAHs in food, and most particularly, as marker of occurrence and concentration of the carcinogenic PAHs

→ However, appropriateness of using BaP was proved dubious

→ The EU actually recommends monitoring the 16 EU-priority PAHs

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PAH intake in the human diet

Contribution from food groups

	UK diet (1983)	
	BaP	Total PAHs
Oils & fats	50%	34%
Cereals	30%	31%
Vegetables	8%	12%

	UK diet (2002)	
	BaP	Total PAHs
Cereals	24%	35%
Vegetables	12%	13%
Beverages	28%	8%
Milk & dairy products	12%	9%
Oils & fats	6%	3%

→ Unprocessed meat: marginal route (always<5%)

Dietary intake of BaP

→ Mean dietary intake of BaP: 50 to 290 ng/day for adults

→ Maximum dietary intake of BaP: 420 ng/day

↓

5-6 orders of magnitude lower than the daily doses observed to induce tumours in experimental animals

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EU regulations for PAHs and PBDEs in food

PAHs : First EU regulation in 2006

2 ng/g for BaP (highest carcinogenic potential) in oils and fats intended for direct human consumption or use as an ingredient in food (Com. Reg. EC No 1881/2006).

→ however, more restricitve national regulations :

- Spain, Italy, Greece, Portugal and Germany : 5 ng/g for the sum of 8 heavy PAHs in olive pomace oils
- Germany : 25 ng/g for total PAHs

→ nothing for meat (except fish : 5 ng/g BaP) or for feedstuffs for animal nutrition

PBDEs : No EU regulation

→ however, EFSA recommendation :

- priority substances to be assessed in the next future
- inclusion of BDE congeners 28, 47, 99, 100, 153, 154, 183 and 209 in EU monitoring programme for feed and food.

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Levels of PAHs and PBDEs in fats and feed :

WP1 & WP4 conclusions

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PBDE contents in oils/fats

Never detected, except in fish oils (2 – 12 ng/g for the sum of the congeners BDE 47, 99, 119, 153)

Never detected, in all kinds of samples from the Dioxin trial (total BDEs <2 ng/g for oil, feed, meat & liver)

Never detected in oils and raw ingredients of feeds from the PAH trial

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PAH contents in oils/fats

WP1	Total [PAHs] (ng/g) : min - max (median) levels				
FAT category	LMW-PAHs	MMW-PAHs	HMW-PAHs	Total PAHs	Samples with [BaP] > 2 ng/g
ANFA	1 - 25 (2)	1 - 90 (15)	1 - 54 (1)	<10 - 107 (18)	3/36
AOCHE	1 - 1 540 (304)	16 - 4 040 (1043)	1 - 340 (20)	21 - 4989 (1022)	15/25
AOPHY	10 - 5 990 (193)	60 - 24 520 (864)	1 - 190 (25)	51 - 30700 (521)	11/16
EBE	149 - 155 (152)	447 - 466 (457)	8 - 56 (32)	610 - 672 (641)	2/2
FACS	25 - 102 (34)	308 - 447 (355)	14 - 61 (16)	356 - 610 (396)	0/3
FISH	7 - 43 (20)	10 -160 (33)	1 - 9 (3)	<10 - 177 (61)	0/9
HYBY	15 - 38 (23)	7 - 280 (230)	3 - 24 (7)	56 - 318 (269)	1/6
LECI	1 - 26 (10)	5 - 140 (20)	1 - 8 (4)	<10 - 155 (31)	0/9
MIX	1 - 240 (17)	8 - 1 080 (18)	1 - 62 (8)	19 - 1394 (65)	1/10
RECY	11 - 136 (25)	2 - 243 (37)	1 - 103 (7)	28 - 482 (61)	2/8
WP4	Total [PAHs] (ng/g)				
AOCHE (PAH trial)	1 530	3 300	470	5 300	[BaP] = 90 ng/g !

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WP1		ΣPAHs (ng/g)	% LMW-PAHs	% MMW-PAHs	% HMW-PAHs	% carcinogenic	% BaP / Σcarcino	% BaP / ΣPAHs	% TEF(BaP) / TEF(carcino)
		Primary production : co-products							
ANFA	Mean	<38	19%	54%	27%	low	9%	2.9%	47%
(n=36)	Median	<27	18%	55%	24%		8%	2.2%	33%
FISH	Mean	74	34%	56%	10%	10%	10%	1.3%	78%
(n=9)	Median	62	34%	53%	8%	9%	11%	1.0%	66%
LECI	Mean	68	23%	61%	16%	20%	9%	1.8%	54%
(n=9)	Median	64	23%	64%	13%	15%	9%	1.7%	44%
AOCHE	Mean	203	25%	69%	6%	10%	9%	0.9%	103%
animal (n=2)	*Std. Dev.*	*114*	*21%*	*21%*	*0%*	*1%*	*3%*	*0.4%*	*58%*
AOCHE	Mean	1006	22%	71%	7%	11%	7%	0.9%	83%
vegetal (n=14)	Median	919	20%	73%	3%	7%	7%	0.4%	84%
AOCHE	Mean	4198	15%	83%	3%	13%	5%	0.4%	69%
tropical (n=3)	Median	4731	16%	83%	2%	10%	6%	0.3%	92%
AOCHE	Mean	2202	26%	70%	4%	9%	7%	0.6%	99%
olive (n=6)	Median	2227	23%	72%	2%	9%	6%	0.5%	75%
AOCHE	Mean	1612	22%	72%	5%	11%	7%	0.8%	87%
TOTAL	Median	1022	18%	74%	3%	9%	7%	0.4%	78%
AOPHY animal	AOPHY 14	91	17%	69%	13%	18%	12%	2.1%	114%
AOPHY	Mean	1047	17%	76%	7%	14%	7%	1.2%	87%
vegetal (n=5)	Median	658	16%	75%	4%	10%	7%	0.5%	71%
AOPHY	Mean	5418	17%	77%	5%	8%	6%	0.7%	79%
olive (n=11)	Median	819	14%	80%	2%	5%	5%	0.5%	69%
AOPHY	Mean	3992	17%	77%	6%	10%	7%	0.9%	83%
TOTAL	Median	677	16%	78%	4%	7%	5%	0.5%	69%
		Secondary production : technical lipids							
FACS	Mean	460	12%	82%	6%	11%	8%	0.9%	110%
(n=3)	Median	397	13%	83%	4%	9%	8%	0.8%	104%
HYBY	Mean	228	19%	75%	6%	11%	5%	0.6%	49%
(n=5)	Median	281	14%	82%	6%	12%	6%	0.7%	41%
MIX	Mean	336	23%	64%	12%	15%	6%	1.0%	39%
(n=10)	Median	98	22%	66%	10%	15%	5%	0.7%	31%
		Waste materials (not used in EU)							
RECY	Mean	119	34%	50%	16%	22%	9%	2.1%	76%
(n=8)	Median	64	31%	52%	12%	18%	9%	1.4%	52%
EBE	Mean	643	24%	71%	5%	19%	6%	1.2%	91%
(n=2)	*Std. Dev.*	*42*	*2%*	*3%*	*5%*	*2%*	*4%*	*0.9%*	*73%*
						10 - 20 %	5 - 12 %	0 - 2 %	50 - 110 %

* Both genotoxic and carcinogenic PAHs: BaA, Chrys, BbF, BkF, BaP, IP, DahA and BP (2005/108/EC).
TEF (toxic equivalent factors): BaP=1, DahA=1, BaA=0.1, BbF=0.1, BkF=0.1, IP=0.1, Chrys=0.01, BP=0.01

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PAH contents in animal feeds (PAH trial)

FEED INGREDIENTS	Σ PAHs (ng/g)	Σ carcino * (ng/g)	% carcino	Animal
Alfalfa hay	41	4.7	11%	rabbit
Barley	10	0.4	4%	
Beet pulp	29	6.0	21%	
Sunflower meal	86	7.6	9%	
	166	19	11%	
Corn	10	0.2	2%	chicken
Full-fat soybean	50	3.4	7%	
Soybean meal	19	2.2	11%	
	80	6	7%	

		RABBIT FEED			CHICKEN FEED		
		Σ PAHs (ng/g)	Σ carcino * (ng/g)	% carcino	Σ PAHs (ng/g)	Σ carcino * (ng/g)	% carcino
INGREDIENTS (with % composition)		41	5	12%	14	1	7%
FEED	High	159	24	15%	317	48	15%
(expected levels)	Med	80	12	15%	159	24	15%
(3% added fats)	Low	0	0		1	0	
FEED	High	153	25	17%	274	51	19%
(determined levels)	Med	81	14	18%	153	30	20%
	Low	32	5	17%	13	2	18%

* Both genotoxic and carcinogenic PAHs: BaA, Chrys, BbF, BkF, BaP, IP, DahA and BP

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Transfer rate of PAHs from feeds to animal tissues

Oils and feeds

PAH trial (high level)

[PAH] ng/g	Total PAHs	BaP	Carcinogenic
Oil (AOCHE)	5300	90	800 (15%)

[PAH] ng/g	Total PAHs	BaP	Carcinogenic
Rabbit feed	150	2.5	25 (17%)
Chicken feed	300	6	50 (17%)

SPIKE trial

[PAH] ng/g	Total PAHs	BaP	Carcinogenic
DP2 (1/2 max WP1 oil)	900	200	400 (40%)
DP3 (max WP1 oil)	1900	400	800 (40%)
DP4 (2 max WP1 oil)	3900	800	1600 (40%)

Animal tissues

Meat & liver	Plasma
< LOD (0.1 to 4 ng/g)	< LOD (0.1 to 1 ng/g)

The transfer rate of PAH from feed to animal tissues was totally inexistent, even when animals were exposed to extremely high levels, regarding the high capability of the animals to quickly metabolize PAHs. Consequently, eggs also will normally not contain significant contents.

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Levels of PAHs and PBDEs in animal tissues and rate of transfer from feed

Part A - WP4 conclusions

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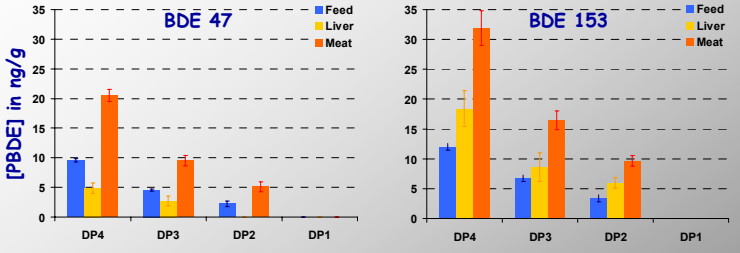
Transfer rate of PBDEs from feeds to animal tissues (SPIKE trial)



	BCF (BDE 47)		BCF (BDE 153)	
Level	Liver	Meat	Liver	Meat
DP4	0.5	2.2	1.5	2.7
DP3	0.6	2.1	1.3	2.4
DP2		2.3	1.8	2.9
DP1				

	Transfer rate in liver (%)		Transfer rate in meat (%)	
Level	BDE 47	BDE 153	BDE 47	BDE 153
DP4	0.6	1.9	21	26
DP3	0.8	1.6	21	25
DP2	0	2.3	23	29

Remark : Transfer rates calculated on a dry weight basis and not on lipid contents of tissues

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Transfer rate of PAHs from feeds to animal tissues

PAH trial (150-300 ng/g in feeds) & SPIKE trial (1000-4000 ng/g in feeds)

Meat & liver	Plasma
< LOD (0.1 to 4 ng/g)	**< LOD** (0.1 to 1 ng/g)

The transfer rate of PAH from feed to animal tissues was totally inexistent (even at high exposure levels to PAHs)

→ **PAHs are not of real relevance as pollutants in feeding fats** (in term of parent compound contents in animal)

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PAH contents (PAH and SPIKE trial)

PAH contents in feed

	PAH trial					
	RABBIT			CHICKEN		
[PAH] ng/g ww	High	Medium	Low	High	Medium	Low
[N, Ace, Acy, Fe]	41	16	5	72	35	5
[Phe, A, Fluo, Pyr, BaA, Chrys]	98	58	24	168	99	6
[BF, BeP, BaP, Per, DA, IP, BP]	14	8	3	31	20	2
TOTAL	153	81	32	271	153	13

	SPIKE trial			
	CHICKEN			
[PAH] ng/g ww	DP4	DP3	DP2	DP1
Phe	837	405	187	<2
Fluo	826	408	192	1.1
Chrys	860	423	196	0.5
BeP	613	310	153	0.2
BaP	811	403	190	0.2
TOTAL	3947	1949	918	1.9
C theo (each)	1200	600	300	0.0

PAH contents in meat & liver (& plasma)

PAH & SPIKE trials	
RABBIT & CHICKEN	
[PAH] ng/g dw	Meat & Liver
N	nd
Acy	<0.1
Ace	<0.1
Fe	<0.1
Phe	<4.0
A	<0.1
Fluo	<0.5
Pyr	<2.0
BaA	<0.1
Triph + Chrys	<0.1
BbF + BkF	<0.1
BeP	<0.1
BaP	<0.1
Per	<0.1
IP	<0.1
DahA + DacA	<0.1
BP	<0.1
TOTAL	<8

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PAH metabolites (OH-PAHs) contents in animal tissues

Oils and feeds

PAH trial (high level)

[PAH] ng/g	Total PAHs	BaP	Carcinogenic
Oil (AOCHE)	5300	90	800 (15%)

[PAH] ng/g	Total PAHs	BaP	Carcinogenic
Rabbit feed	150	2.5	25 (17%)
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SPIKE trial

[PAH] ng/g	Total PAHs	BaP	Carcinogenic
DP2 (1/2 max WP1 oil)	900	200	400 (40%)
DP3 (max WP1 oil)	1900	400	800 (40%)
DP4 (2 max WP1 oil)	**3900**	**800**	**1600 (40%)**

Animal tissues

OH-PAHs (naphthol, phenathrenol and fluoranthenol isomers, and 1-chrysenol, 1-pyrenol and 3-benzo[a]pyrenol) determined in plasma, bile and rabbit urine

- All metabolites detected in bile and in rabbit urine (except 1-chrysenol and 3-benzo[a]pyrenol)
- Metabolites contents clearly significant for the high level treatment
- PAH metabolites not detected in plasma (even for the high exposure level)

- All metabolites detected in chicken bile and plasma (including chrysenol & benzo[a]pyrenol)
- Metabolites contents clearly significant for the high level treatment (bile and plasma)
- presence of metabolites in plasma: potential biological impact by PAH exposure

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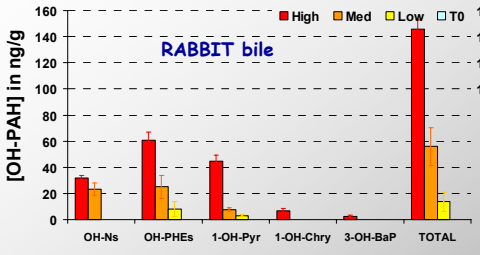
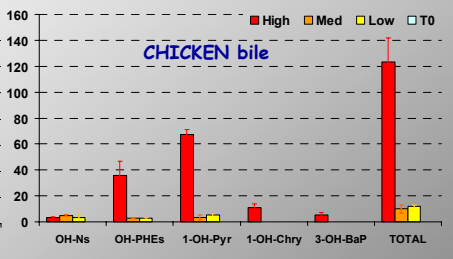
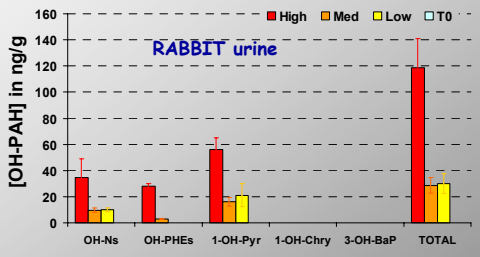
PAH metabolite (OH-PAHs) contents (PAH trial)







CHICKEN & RABBIT plasma

PAH metabolites not detected

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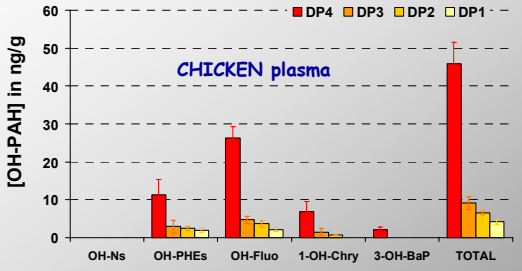
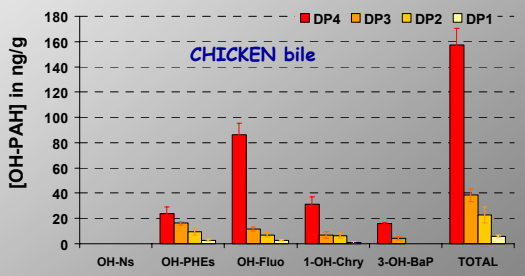
PAH metabolite contents (SPIKE trial)





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Levels of PAHs and PBDEs in animal tissues and rate of transfer from feed :

B/ Proposal of a control program

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Conclusions

BDEs :

→ as for PCBs, BDEs are greatly bioaccumulated in animal tissues, if present in feeds : case of feeding fats using contaminated fish oils

→ Marginal risk for animal health and for the consumer of the meat

PAHs :

→ No transfer to animal meat, even when using highly contaminated oils from chemical or physical refining : Use of PAH metabolites as biomarkers of PAH exposure

→ At extremely high PAH levels in feeds, presence of metabolites in plasma : potential biological impact ?

→ No effect of high or extremely high PAH contents in feeds on growth and health performances of animals

→ No risk for animal health and for the consumer of the meat

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Main conclusions and opening questions

PAHs in animal tissues :

No transfer to animal meat, even with highly contaminated feed

→ Use of PAH metabolites as biomarkers of PAH exposure

PAH metabolites :

Mainly a qualitative approach (hard to quantify, need for normalized evaluation)

No representative of the physiological state of animals

but if present in plasma (body circulation): potential biological impact

So why not follow them in meat ?: still an analytical challenge !

PBDEs in animal tissues :

As PCBs, bioaccumulated

PBDE trasnfert to animal meat correlated to PBDE contents in feed

Evaluation of transfer rate : need for additional data, such as mass of feed ingested, animal body weight …

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Proposal (1/4)

→ PAHs contamination of feedingstuffs is unlikely to represent a health hazard to animals and to humans from the consumption of animal products, and thus the utility of controlling PAH in feeding fats could be considered as negligible, BUT :

As setting a threshold limit below which toxicity of PAHs could be considered negligible is difficult, the presence of PAH in foodstuffs should be reduced to as low as reasonably achievable (ALARA principle) by controlling environmental PAH contamination of fats/oils & basal ingredients for feeds and all procedures that could cause PAH contamination during feeds and food processing.

→ PBDEs: limited food & toxicological data are available, but new data and EU-regulation/recommendation can be expected in the near future.

The analysis of co-products for the presence PBDEs (when available methods) seems to be the only possibility of prevention.

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Proposal (2/4)

→ PAH contamination by atmospheric deposition : rinsing the seeds with organic solvent in an ultrasonic bath ?

→ Avoid contact of seeds wit mineral oil residues (e.g. transport in jute bags) and contact with recycled polyethylene films (oil packaging)

→ PAH contamination by drying/heating processes : refining can drastically reduce the PAH contents in oils.

Methods suitable to avoid or remove PAHs from oils (from Deliverable 9):

Co-product	Origin of contamination	Methods to avoid contamination	Actual situation in the industry	Methods to remove contaminants from co-products	Costs for contaminants removing process
AOCHE and AOPHY	Drying of seeds / nuts / through direct flame before oils extraction process	Drying materials avoiding contact with exhaust. Use indirect heated air flow	The general trend is to replace the direct flame process with hot air drying	Filtration using 1 – 3% of active carbons	From 10 to 20 Euros / ton. This cost has been estimated considering the usual filtration costs in oil processing industry including the extra-costs of active carbons

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Proposal (3/4)

PAH contamination by drying/heating processes :

→ The refining process normally involves degumming, neutralization, partial elimination of waxes, decolouration and deodorization. The decolouration step has been amended to include the use of activated carbon together with activated clays, which reduces significantly the levels of PAHs in the oil.

→ The deodorization process seems to have little effect on high molecular PAHs and removes mainly light PAHs (up to four aromatic rings), while higher condensed heavy PAHs are mainly removed by charcoal treatment.

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Proposal (4/4)

Risk assessment of PAH in food : the use of BaP as marker of PAH contamination in food and of the benzo[a]pyrene toxic equivalency factors (TEFs) do not adequately describe the presence and potency of PAH mixtures

→ Monitoring now the EU-priority PAHs (both genotoxic & carcinogenic; benzo[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, cyclopenta[cd]pyrene, benzo[a]pyrene, dibenz[ah]anthracene, dibenzo[ae]pyrene, dibenzo[ah]pyrene, dibenzo[ai]pyrene, dibenzo[al]pyrene, Indeno[1,2,3-cd]pyrene and 5-methylchrysene + benzo[c]fluorene), instead of the usual EPA PAHs

→ Monitoring also alkylated & nitro-PAHs (present in vegetal oils, potentially more toxic than PAHs and still ignored by legal regulations)